International Journal of Pharma and Bio Sciences V1(2)2010

ANTICONVULSANT ACTIVITY OF FLOWER PART OF N. ODERUM

POOJA SAINI*[1], N. KANNAPAN[3], ANUPAMA DIWAN[4], PARVEEN KUMAR[2], VISHAL ANTIL[2], SHREYA SHARMA[2] AND SANDEEP SINGH[4]

[1]R.K.S.D.College of Pharmacy, Kaithal, India
[2]G.V.M. College of Pharmacy, Sonipat, India
[3]Annamalai University, Chidambaram, TamilNadu, India
[4]Hindu College of Pharmacy, Sonipat, India

Corresponding author poojasaini1985@gmail.com

ABSTRACT

Different extracts (Petroleum ether, methanolic, aqueous) of *Nerium oderum* at a dose level of 400 mg/kg were screened for the anticonvulsant activity using Maximum Electroshock Induced Seizures & Pentylene Tetrazole Induced Seizures test models. From the present study, Petroleum ether extract of *N. oderum* showed better anticonvulsant activity as compared to other extracts.

KEY WORDS

Nerium oderum, Anticonvulsant, Pentylene Tetrazole Induced Seizures and Maximum Electroshock Induced Seizures

INTRODUCTION

Nerium oderum commonly known as "kaner" belonging to family "Apocynaceae" is provoked as a toxic plant but traditionally is being used to cure various ailments such as asthma, corns, cancer and epilepsy. A wide spectrum of biological activities have been reported with various constituents isolated from different parts of the plant. Two varieties are found in the plant one with the white flowers (*Nerium indicum*) and one with the pink flowers (*Nerium oderum*). This is an erect, smooth shrub 1.5 – 3 meters in height and contains a cream colored, stick, resinous juice. The leaves are mostly in whorls of 3 to 4, linear lanceolate. The fruit is cylindrical, in pairs with deep linear striations, slightly twisted & 15-20 centimeters long. The flowers are showy sweet scented, single or double 4-5 centimeters in diameter, white, pink or red and borne on terminal inflorescences (Cymes). Root, bark and seeds contain cardiac glycosides that have a paralyzing action on the spinal cord. Oleandrin, a pure component from the plant has a stimulating action on the heart and also a pronounced diuretic effect. The alcoholic extract shows antibacterial activity and oil obtained from the root is used in leprosy and skin diseases [1]. The plant is recognized in folk medicine as antidote, antibacterial, antileprotic, anticancer, cardiotonic and C.N.S. depressant [2-4]. In ethno botanical literature, it is mentioned to be effective in the treatment of cancer, corns & epilepsy and also used as C.N.S. depressant but no scientific data is reported. Therefore, in the present study the flowers of the plant are screened for anticonvulsant activity.

MATERIALS AND METHODS

Collection of Plant Material & Preparation of the Plant Extract

The flowers of *Nerium oderum* were collected

ANTICONVULSANT ACTIVITY OF FLOWER PART OF N. ODERUM

and authenticated by Dr. Gyanendra Tiwari. A voucher specimen was deposited in the Department of Pharmacognosy, B.R. Nahata College of Pharmacy, Mandsaur, India. The collected flowers were dried; powdered and successive solvent extraction was done with Petroleum ether (60-65° C), methanol and water. The solvents (Petroleum ether, methanol and water) were recovered by the distillation process. Percentage yields (% w/w) of different extracts of *N. oderum* obtained by successive solvent extraction were determined and found to be 4.424, 55.487, 25.44 for petroleum ether, methanol and aqueous respectively. These three extracts were then tested for the anticonvulsant activity.

Experimental Animals

Wistar rats and Male Swiss albino mice were obtained from Animal House unit of the Department of Pharmacology, B.R. Nahata College of Pharmacy, Mandsaur (M.P.). The animals are housed in groups of 5-6 under standard laboratory conditions (temp. 25±2°C, relative humidity 55±5% and lighting 08:00-20-00 hr.) with food & water ad libitium.

Acute toxicity study

Acute oral toxicity study was performed as per the Organization of Economical Cooperation and Development (OECD) 425 guidelines [5] .Wistar strain albino rats of either sex were used for the study. The animals were kept on fasting overnight and were provided only water, after which the extracts (Petroleum ether, Methanol, aqueous) were administered orally at the dose level of 2000mg/ Kg and observed for 14 days. If no mortality was observed, then the extracts were found to be safe.

Assessment of anticonvulsant activity by Maximum electroshock (MES) induced seizures [6, 7, 8]

Animals are divided into 5 groups, each group consisting of 5 animals. Control group received 1 % Tween solution whereas standard group received Phenytoin 20 mg/Kg i.p. and remaining groups received the test drug at a dose of 400 mg/Kg. After 60 min., electroshocks (150 mA for 0.2 sec.) were applied by means of stainless steel pinna electrodes. The extensor phase of convulsion process was observed.

Assessment of anticonvulsant activity by Pentylene tetrazole (PTZ) induced seizures [6, 7, 8]

Mice of either sex with a body weight between 18 and 22 g have been used for the study. Control group received 1 % Tween solution whereas the standard group received Diazepam 4 mg/Kg i.p., remaining groups received the test drug at a dose level of 400 mg/Kg. The test compound was given orally to groups of 5 mice. After 30 min 60 mg/Kg PTZ (Metrazol) was injected i.p. Each animal was placed into an individual plastic cage for observation lasting 1 h. Seizures and tonic-clonic convulsions have been recorded.

Statistical analysis

Data obtained from pharmacological experiments are expressed as mean ± S.E.M (Standard Error mean). Difference between the control and the treatments in these experiments was tested for significance using ANOVA followed by Dunnett's test. All statistical analysis was performed with prism 4.0 (Graph pad software Inc., San Diego, CA). $P < 0.05$ was considered significant.

RESULTS

Acute toxicity studies depicted no mortality upto the dose level of 2000 mg/Kg body weight. So, the extracts can be considered safe for long term administration. In PTZ induced seizure test, the onset of convulsions in control group was observed at 130.6 sec. after PTZ injection. The test drug delayed the onset of convulsions to 170.8 sec. (*N. oderum*, pet. ether extract) (Table-1). In supra Maximal Electroshock Seizure (MES) test, it was observed that the test drug (*N. oderum*, Petroleum ether extract) produced a

significant reduction in the duration of extensor phase which was reduced to 6.6 sec. (P< 0.05), while in control group this duration was 16.20 sec. (Table 2)

Table 1.
Anticonvulsant activity of different extracts of N. oderum by P.T.Z. induced seizures

S.No.	Treatment	Onset of convulsion (sec.)	Protected animals (%)
1	Control	130.6 ± 4.02	20
2	Standard	187.0 ± 2.56**	100
3	Pet. Ether	170.8 ± 2.74**	100
4	Methanol	156.4 ± 4.02**	80
5	Aqueous	166.4 ± 3.31**	80

Values are expressed in mean ± S.E.M., n = 5, ** Significant at p < 0.01 Vs control, * Significant at p <0.05 Vs control Dunnet's test, dose of extracts = 400 mg/Kg, Standard (Diazepam) = 4 mg/Kg

Table 2.
Anticonvulsant activity of different extracts of N. oderum by M.E.S. induced seizures

S.No.	Treatment	Duration of tonic hind limb extension(sec.)	Incidence of convulsions in no. of mice
1	Control	16.20±0.86	2/5
2	Standard	9.60±0.50**	5/5
3	Pet. Ether	6.6±0.50**	4/5
4	Methanol	12.40±0.92*	2/5
5	Aqueous	11.60±0.50*	3/5

Values are expressed in mean ± S.E.M., n = 5, ** Significant at p < 0.01 Vs control, * Significant at p <0.05 Vs control Dunnet's test, dose of extracts = 400 mg/Kg, Standard (Phenytoin) = 20 mg/Kg

DISCUSSION AND CONCLUSION

Acute toxicity studies indicate that the flower extract of *N. oderum* may be safely used in the animals upto the dose level of 2000 mg/ Kg body weight. The Petroleum ether extract of *N. oderum* significantly inhibited the PTZ and MES induced convulsions. It was effective against MES induced seizures, since inhibition of the MES test predicts activity against generalized tonic-clonic seizures. *N. oderum* (Petroleum ether extract) was active against MES induced seizures. PTZ is the most frequently used substance as well as an acute experimental model in the preliminary screening to test potential anticonvulsant drugs. Several biochemical hypothesis have been advanced involving the inhibitory GABAergic system and the system of the excitatory

ANTICONVULSANT ACTIVITY OF FLOWER PART OF N. ODERUM

amino acid glutamate and aspartate [9].The mechanism by which PTZ is believed to exert its action is by acting as an antagonist at the $GABA_A$ receptor complex [10]. Drugs protecting against tonic-clonic seizures induced by PTZ are considered to be useful to control myoclonic and absence seizures in humans [11]. In this study's experiments, the extract of *N. oderum* replicated the effect of this anti epileptic drug by delaying tonic convulsion and mortality. The benzodiazepine site in the $GABA_A$ receptor and T-type Ca^{2+} currents could be targets for future studies to know the mechanisms of action of *N. oderum* (Petroleum ether) extract. It suggests that *N. oderum* is useful in suppressing generalized tonic-clonic seizures. Anticonvulsant activity of *N. oderum* in inhibiting seizures may be by regulating GABA mediated synaptic inhibition through action at distinct sites of the synapse. Summarizing the data obtained in this study, the results suggest a possible anticonvulsant effect of *N. oderum*.

REFERENCES

[1] B. L. Manjunath. The Wealth of India, Council of scientific and industrial research, New Delhi. 1: pp. 15-17 (1948).

[2] R. N. Chopra, S. L. Nayar, I. C. Chopra. Glossary of Indian Medicinal plants, Council of Scientific and Industrial Research. pp. 175 (1956).

[3] A. Zia, S. Siddqui, S. Begum, S. Suria. Journal of Ethnopharmacology. 49:33 (1995).

[4] K. R. Kritikar, B. D. Basu, M. P. Sing. Indian medicinal plants India, Dehradun. pp. 1584 (1975).

[5] OECD guidelines for testing of chemicals, acute toxicity studies-fixed dose procedure, Proposal for a new guideline 425, version. 1, 14 (2004).

[6] S. K. Kulkarni. Handbook of Experimental Pharmacology. pp. 133 (2005).

[7] G. S. Achilya, S. G. Wadodkar, A. K. Dorle et al. Indian journal of Pharmacology. pp. 33-36 (2005).

[8] H. G. Vogel. Drug discovery and evaluation. 2nd ed., pp. 422, 487 (2002).

[9] R. I. McDonald, K. M. Kelly. Antiepileptic drugs: Mechanisms of action. Epilepsia 34: S1-8-20 (1993).

[10] R. Ramanjaneyulu, M. K. Ticku. Interactions of pentamethylenetetrazole and tetrazole analogues with thepicrotoxinin site of the benzodiazepine-GABA receptor ionophore complex. Eur.J.Pharmacol. 98: 337-345 (1984).

[11] W. Loscher, D. Schmidt. Which animal's models should be used in the search for new antileptic drugs? A proposal based on experimental and clinical consideration. Epilepsy Res 2: 145-181 (1988).